REMARKS

Claims 1-11 remain in this application. Please add claim 12.

The present invention provides a novel system allowing detection and/or efficient purification of biomolecules and/or proteins expressed at low level, preferably in their natural hosts. The biomolecule and/or protein complexes that are detected and/or purified exhibit their natural acitivity, i.e. they are present in the form of their functional complexes. The presence of the native, functional form of a subunit of a complex is of great importance because the subunit will only exhibit its binding characteristics to other subunits or biomolecules when in its native form.

Further, the inventive method advantageously enables the generation of highly purified biomolecule and/or protein complexes, even where the nucleic acids for the corresponding proteins of interest are expressed at low levels. This efficient method allows detection of proteins at their basal level.

It was not known previously that a combination of two affinity tags could be used for this purpose. The combination of tags required for this new application was not known and previous publications did not reveal that the combination disclosed would be successful. Accordingly, the inventive method involves two different affinity purification steps. The inventive method provides a very efficient and at the same time very gentle purification which results in pure biomolecules and/or proteins being freed from remaining contaminants and other unwanted substances.

Enclosed is a schematic illustration of exemplary embodiments of the inventive method. According to claim 1 and looking to the left-hand column of the illustration, an exemplary protein complex comprising two subunits is purified according to the invention, wherein one of the subunits of the protein complex is fused to two different affinity tags. First, in step (a), an expression environment is provided, which contains a nucleic acid coding for the subunit being fused to two different affinity tags as well as a nucleic acid coding for the other subunit of the protein complex. In step (b), the expression environment provides for the expression of both subunits of the protein complex. One of the expressed subunits is a fusion protein comprising the two different affinity tags. In step (c), two different affinity purification steps are carried out to obtain a pure protein complex. In step (d), the pure protein complex of step (c) is detected by means of gel electrophoresis.

According to claim 2 and looking to the right-hand column of the illustration, an exemplary protein complex with two subunits is purified, whereby each subunit is fused to one different affinity tag. In step (a), an expression environment is provided, which contains two nucleic acid sequences, each coding for a subunit of the protein complex being fused to different affinity tags. In step (b), the expression environment provides for the expression of both subunits of the protein complex.

Both of the expressed subunits are fusion proteins, with each subunit comprising one different affinity tag. Method steps (c)–(d) are the same as described above.

The method of the invention as described above can also be used for purifying complexes having more than two subunits. Likewise, complexes having one subunit and a biomolecule can be purified. Examples of this are antigen-antibody complexes as well as a complex of a DNA binding protein and DNA.

Paragraph 3 and 4: Rejection under 35 USC 112, First Paragraph

In order to satisfy the enablement requirement of 35 U.S.C §112, first paragraph, the specification must teach one of skill in the art to make and use the invention. That some experimentation is needed, does not preclude enablement as long such experimentation is not undue. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. The claims includes the following elements.

a) providing an expression environment containing one or more heterologous nucleic acids encoding one or more subunits of a biomolecule complex;

One of ordinary skill in the art would clearly understand how to provide an expression environment. One of ordinary skill in the art is taught that any host cell can be used, including prokaryotic, yeast, or mammalian cells (page 6, lines 16-19). The use of natural host cells is preferred (page 6, lines 20-21). At the time of the invention, numerous expression systems were known in the art, including E. coli, mammalian, viral, and plant expression systems. Expression systems were also known in the art to be used in the production of proteins having a tag.

The following documents illustrate that numerous expression systems existed in the art at the time of the invention.

Sun et al., Arch. Biochem. Biophys. 1997 Sept. 1(1): 135-42.

Kubler et al., Int. J. Oral Maxillofac. Surg. 1998 Aug. 27(4): 305-09.

Soen, et al., J. Muscle Res. Cell Motil. 1998 Aug. 19(6): 639-46.

Pei et al., Protein Expr. Purif. 1998 Jul. 13(2): 277-81.

Takahashi et al., Blood. 1998 Apr. 15; 91(8): 2830-8.

Hellwege et al., FEBS Lett. 1998 May 1; 427(1): 25-8.

Austin et al., Protein Expr. Purif. 1998 Jun; 13(1):136-42.

Schenk et al., Int. J. Biochem. Cell Biol. 1998 Mar; 30(3): 369-78.
Fucentese et al., J. Membr. Biol. 1997 Nov 15; 160(2): 111-17.
Naviaux et al., J. Virology 1996 Aug. 70(8): 5701-5705.

Further, numerous expression vectors are described in the literature and are commercially available. Depending on the biomolecule and host cell of interest, one of ordinary skill could easily purchase or routinely develop an expression vector for a given biomolecule. One of ordinary skill in the art is taught that the heterologous nucleic acid should contain appropriate transcriptional control sequences, such as a suitable promoter, enhancer, or polyA site, so that the nucleic acid can be maintained in the host cell or cell-free system and so that the fusion protein will be expressed at basal levels (page 6, line 10-14; page 7, lines 1-3). For example, the heterologous nucleic acid to be used is determined by the protein complex to be produced. Starting out from a certain biomolecule complex to be detected or purified, a skilled person can easily determine or identify the corresponding nucleic acid. The insertion of a nucleic acid of interest into an expression vector is well known in the art. Hence, providing such an expression environment would be nothing more than following well known directions well within the ordinary skill in this art. Further, there is no requirement that the specification disclose every example covered by a claim.

In addition, multi-subunit biomolecule complexes are well known in the art. The following documents illustrate other biomolecule complexes that one of ordinary skill in the art could express using the methods of the invention.

Machesky et al., J. Cell. Biol. 1994 Oct. ; 127(1): 107-15.
Liu et al., EMBO J. 1998 Feb. 16; 17(4): 1096-1106.
Sacher et al., EMBO J. 1998 May 1; 17: 2494-2503.

(b) maintaining the expression environment under conditions that facilitate expression of the one or more subunits in a native form as fusion proteins with subunits being fused to at least two different affinity tags, wherein one of the affinity tags consists of one or more IgG binding domains of Staphylococcus protein A;

As vectors are described in the literature, so are conditions for their expression. As cited above, numerous expression systems are known in the art for all types of host cells. It is well known that vectors can be provided for the chosen host cell that express biomolecules and/or proteins and that those proteins can be expressed as a fused complex. Again, the expression is routine to one of ordinary skill.

In addition, the following documents illustrate that the methods of the invention have been carried out in non-yeast cells.

Bouwmeester et al., Nat. Cell Biol. 2004 Feb.6(2): 97-105 (especially methods section).

WO 04/035783.

WO 04/031242.

WO 04/009622.

WO 04/009619.

WO 04/007544.

From these numerous documents, it is clear that the cells and conditions, respectively, used in the Examples of the present application are merely one specific embodiment of a plurality of other possibilities and that one of ordinary skill was easily able to make the methods work with other cells and with other conditions.o

(c) purifying the complex by a combination of at least two different affinity purification steps each comprising binding the two or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed to provide a purified biomolecule and/or protein complex;

The use of affinity tags in purification techniques, especially column-based purifying techniques, are well known in the art. Further, the example set forth in the specification (page 16, lines 26 through page 18, line 10) illustrates a purification method. One of ordinary skill in the art would readily recognize that the two-step purification process could be used for plant, mammalian, bacterial, and other yeast biomolecule complexes with few if any changes. The purification method used is the same regardless of what type of biomolecule complex is purified as long as the complex is affinity tagged. Therefore, the specification is enabling for all types of host cells and biomolecule complexes because the method used is the same.

The following document illustrates that only minor modifications would be needed and that these modifications would be obvious to one of ordinary skill in the art. Although the article was published after the priority date, the article demonstrates that only minor changes of the experimental protocol are necessary in order to apply the method to mammalian protein complexes.

Gavin et al., Nature 2002; 415(10): 141-147.

(d) detecting the purified biomolecule and/or protein complex (now dependent claim 12)

Methods of detection making use of affinity tags are well known. One of ordinary skill could easily determine how to detect a biomolecule of interest. The following documents illustrate that the use of tags is well known in the art.

Murray et al., Analytical Biochemistry 1995; 229: 170-79.
Sacher et al., EMBO J. 1998 May 17: 2494-2503.

Paragraph 5: Rejection under 35 USC 112, Second Paragraph

Claims 1 and 2 have been amended to clarify that only a purification step is required.

The Commissioner is hereby authorized to charge any additional fees which may be required in this application to Deposit Account No. 06-1135.

Respectfully submitted,
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